TEMPLATE SPECIFICITY OF THE CYTOPLASMIC DNA POLYMERASE IN XENOPUS LAEVIS OOCYTES

H. PINON, M. BARAT, C. DUFRESNE, J.C. MOUNOLOU

Laboratoire de Biologie Générale, Université Paris-Sud, 91405 ORSAY - FRANCE

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SUMMARY - Various DNAs, with labelled DNA precursors, have been injected in large oocytes of Xenopus laevis. It appears that double stranded mitochondrial DNAs are efficient templates ; on the contrary DNAs from nuclei or bacteria are not.

The method of injecting DNA in oocytes or eggs to get a living assay of the polymerase was developed at first by Gurdon and coworkers (1) and led to the conclusion that "DNA polymerase" activity can be revealed only after maturation (2). In previous experiments we reported that native yeast mitochondrial DNA (Ym-DNA) is a good template for the cytoplasmic DNA polymerase of the immature oocyte (3). These observations suggest then that this Ym-DNA contains some structural property needed to be recognized as a substrate by the polymerase before maturation. Several hypotheses can be put forward to explain the specificity of the oocyte cytoplasmic "DNA polymerase" activity :

- The injected template is very nicked and somehow activated (4).
- The Ym-DNA has a low GC content (18 %) and contains AT rich segments with low genetic complexity (5). These structures may be recognized by the enzyme(s) using such DNA as a template for DNA synthesis.
- The recognition specificity is shared only by mitochondrial DNAs whatever their origin.

We want to present here a more precise study of the Ym-DNA-oocyte system and some elements to discriminate between the previous hypotheses.

MATERIAL AND METHODS

Oocytes - Ovaries are taken off from Xenopus laevis adult females four days after animals have been induced to lay eggs by an in-

jection of human chorionic gonadotrophin (200 I.U). Large follicles are one by one removed and kept in the Barth's medium as modified by Gurdon (6).

DNAs - Three purified yeast DNAs from Saccharomyces cerevisiae have been used : nuclear DNA, mitochondrial DNA from a "grande" strain (density in CsCl : 1,684g/cc) and mitochondrial DNA from the "petite" strain DM1 (density in CsCl : 1,671g/cc) (7). All these DNAs were purified on hydroxyapatite (8) which yields fragments of an average molecular weight 2.10^6 . The two mitochondrial DNAs were kindly supplied by G. Fonty, Clostridium perfringens DNA was purchased from Sigma and is also fragmented. Dictyostelium discoïdeum DNA is a gift from F. Monier. From Xenopus oocytes we extracted twisted circles using ethidium bromide-CsCl gradient (9) ; ethidium bromide is carefully removed with butanol and CsCl by dialysis.

- Labelled ^3H deoxyadenosine is obtained either from the Commissariat à l'Energie Atomique or the Radiochemical Center Amersham.

Incorporation analysis - 6 hours after injection (30nl/oocyte), oocytes are washed, then crushed in cold SSC ; a wide piston-cylinder space is required to avoid follicular cell breakage. The homogenate is centrifuged 5mn at 750g. On part of the supernatant sarkosyl (Geigy), is adjusted to 0.7 % and CsCl to reach a final density around 1.7g/cc ; gradients are performed in a Beckmann model 65 rotor drived 62 hours at 37,000 rpm, then fractionated. The radioactivity of trichloroacetic precipitable material is checked.

RESULTS AND DISCUSSION

The Ym-DNA - large oocytes system

At the end of the oogenesis the germinal vesicle exhibits condensed chromosomes in diploten stage ; they don't incorporate labelled DNA precursors but mitochondria can still incorporate such precursors in their DNA (unpublished results).

In large oocytes, injected with Ym-DNA, labelled deoxyadenosine is incorporated in a Ym-DNA density material. Fig. 1 shows that the bigger the quantity of injected Ym-DNA, the bigger the peak of radioactivity found at the Ym-DNA density in the CsCl gradient. To normalize the results, we define the relative efficiency of incorporation (REI), for the injected DNA compared to the Xenopus one, by the formula :

$$REI = \frac{Ax}{Ab} \cdot \frac{3}{x} \cdot \frac{b}{a}$$ where $\frac{b}{a}$ is the ratio of the radioactivity found in the injected DNA peak to the radioactivity of the Xenopus DNA peak ; this crude result is corrected for the relative amount of template (3ng/oocyte for Xenopus (11) and x ng/oocyte for the injected DNA) and for the relative efficiency of the labelled precursor (Ax is the adenine content of the Xenopus DNA and Ab the same para-

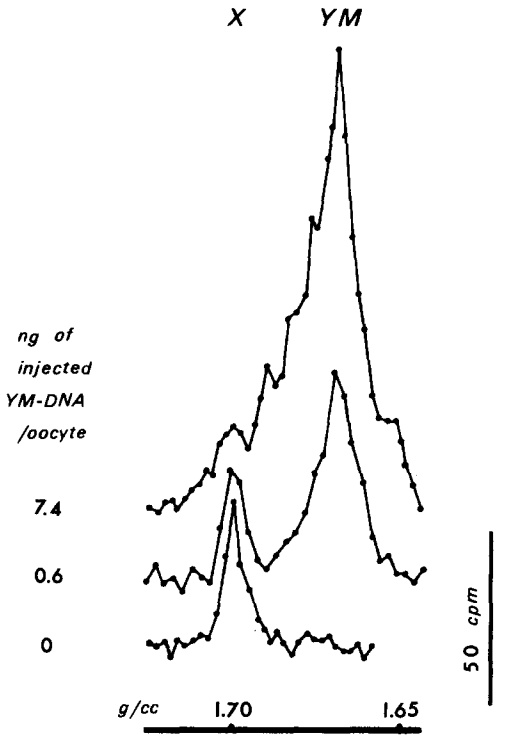

Fig. 1 : Batches of 100 large oocytes have been injected with labelled deoxyadenosine and various concentrations of mitochondrial DNA from the DM1 yeast strain. After analysis (see Material and Methods) incorporated radioactivity is expressed as a function of density in CsCl gradients.

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meter for the injected DNA). For a given DNA :

$$REI = K \frac{b}{a x}$$

No hypothesis is made upon the biological meaning and the relative amount of the two incorporations : the one in the Xenopus DNA and the one in the exogenous DNA. Assuming that the incorporation in the endogenous oocyte DNA is constant, it is used only as a reference. So it becomes possible to compare results obtained with various injected templates.

Injected in large oocytes, Ym-DNA of various densities (1,684 and 1,671g/cc) have the same behavior but two parameters modify their REI.

1) The REI of Ym-DNA decreases from 10-11 in spring (northern hemisphere) to 1-1.5 in autumn.

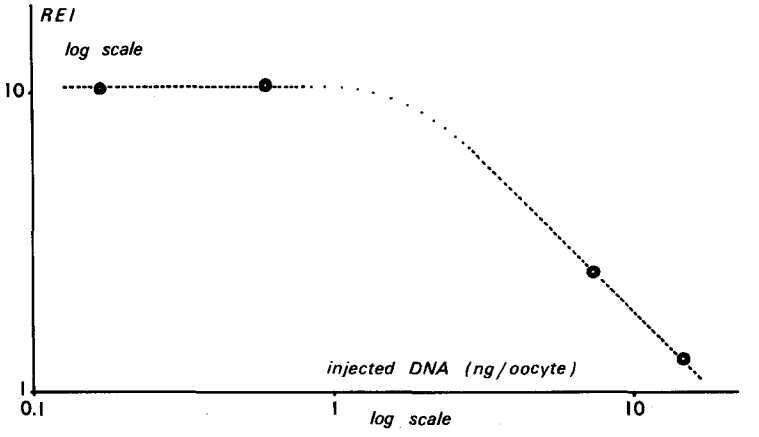

Fig. 2 : REIs for Ym-DNA have been calculated from batches of 100 to 230 injected large oocytes and then expressed as a function of the amount of template injected per oocyte.

Injected DNA			GC content	REI / REI (YM-DNA)
native	eucaryotic nuclear	Saccharomyces cer.	39	0,1*
		Dictyostelium dis.	23	0,1
	eucaryotic mitochondrial	yeast "grande" strain	18	1
		yeast "petite" DM1	4	1
		Xenopus laevis	41	1-2*
		Dictyostelium disc.	28	0.7-1.2
	pro.	Micrococcus lys.	71	0,05
		Clostridium perf.	31	0,15
heat-denatured		yeast "grande" strain	18	0.5-0.7
		yeast "petite" DM1	4	
		Micrococcus lys.	71	

Table 1 : Various characteristics of the injected templates tested are presented with the REIs obtained, compared to the one of native YM-DNA recorded at the same time.
*Not seen by CsCl gradient analysis (see text).

2) Fig. 2 shows the variation of REI as a function of the amount of DNA injected per oocyte. For x values lower than 1ng/oocyte the REI is constant : $\frac{b}{a}$ and x are varying in the same way. But for x values over 3ng/oocyte $\frac{b}{a}$ is constant and the REI is then inversely proportional to x (straight line with a slope of -1 in the log log plot). This fact shows that the tested enzymatic activity is entirely saturated by 3ng of Ym-DNA, amount of DNA unable to saturate the egg activity (11).

Assay of other DNAs

Table 1 summarizes results obtained with various templates. These DNAs differ in their structures (from Xenopus mitochondrial circles to fragments obtained by hydroxyapatite chromatography), their GC contents (from 4 % to 71 %) and their origins (eucaryotic nuclear and mitochondrial, procaryotic). The REIs calculated for each DNA are compared to the ones of Ym-DNA obtained with oocytes of the same female.

Injected DNA	Total radioactivity cpm/oo	ADN cpm/oo	ADN cpm/oo/10^4 total cpm
No	10785	6,3	5,8
	10729	7,1	6,6
yeast nuclear	8806	5,5	6,2
	7942	5,3	6,7
Xenopus mitochondrial	11700	9,3	7,9
	10867	12,2	11,2

Table 2 : Batches of 70 to 80 large oocytes (oo) are injected with labelled deoxyadenosine and either saline buffer or 2.25ng/oocyte of yeast nuclear DNA, or 1ng/oocyte of Xenopus mitochondrial circular DNA. After incubation the 750g oocyte supernatant is treated with sodium dodecyl sulfate (1 %), then Na ClO_4 (1M), then chloroform-isoamyl alcohol (24/1, v/v). The centrifuged clear aequous phase is treated with Na OH (0.5M, 30°C, 16 hours), neutralised before addition of bovine serum albumin (200µg/ml) and checked for the trichloroacetic precipitable radioactivity.

Some comments have to be made for Xenopus mitochondrial DNA, yeast nuclear DNA and Dictyostelium discoideum DNAs. In Table 2 are given results obtained with DNAs of the same buoyant density as the Xenopus one. For this reason REI cannot be measured by reference to the internal incorporation of Xenopus, but by reference to another batch of oocytes injected only with labelled deoxyadenosine. Even if this method is less precise, the results are clear : yeast nuclear DNA cannot be used as a template by the large oocyte in the contrary to the Xenopus mitochondrial DNA prepared from twisted circles. Fig. 3a shows the buoyant density pattern of Dictyostelium discoideum DNA, as seen by analytical centrifugation. According to Firtel and Bonner (12) two major components are observed : nuclear DNA and mitochondrial DNA. After injection only the mitochondrial component seems to incorporate deoxyadenosine (Fig. 3b) despite that the nuclear DNA has a higher AT content.

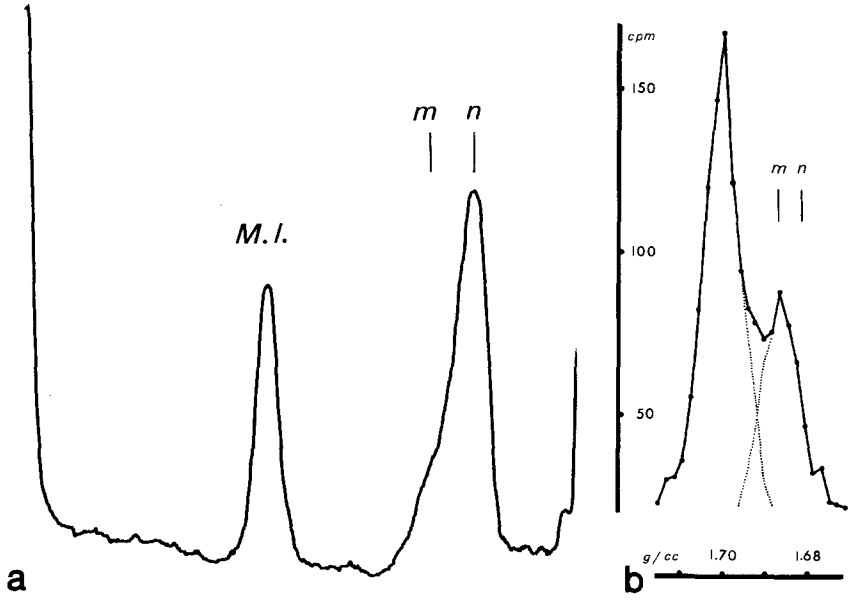

Fig. 3a : In analytical centrifuge, Dictyostelium discoideum DNA exhibits a shoulder of mitochondrial DNA (m) on the heavy side of the nuclear DNA (n), (here a little less than 25 % of the total). Micrococcus lysodeikticus DNA (M.l.) is used as reference.

Fig. 3b : After injection only the mitochondrial component (m density) incorporates labelled deoxyadenosine.

The biological meaning of the high REIs observed with heat denatured DNAs is still unknown. It could be related with the general occurence of single strands that the "DNA polymerising" enzymes can recognize.

Nevertheless it appears that the ability of a native DNA to be used as template is not related to a low GC content but to its origin. Mitochondrial DNAs give the highest REIs even when they have been purified without nicks as seen for the twisted circles of the Xenopus mitochondrial DNA. We propose that this activity, located in the cytoplasm of the oocyte and different from the one found in the egg by its amount, its isolectric point (13) and its template specificity, is related to the mitochondrial polymerase.

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